STUDIES ON *CORTICIUM ROLFSII* (SACC.) CURZI (*SCLEROTIUM ROLFSII* SACC.).

I. CULTURAL CHARACTERS AND PERFECT STAGE. II. MECHANISM OF PARASITISM.

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(Plate v; seven Text-figures.)

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Sclerotium rolfsii Sacc. was first recorded in New South Wales in 1902 (Noble *et al.*, 1934), and since then has resulted in severe losses in many crops. It is an extreme facultative parasite, causing a crown and root rot of a large number of plants, Weber (1931) listing over 150 known hosts.

The terminology of this species is somewhat confused. Owing to the difficulty of inducing sporulation, the appearance and size of the sclerotia and other vegetative characters are generally used in classification. *S. rolfsii*, for example, is distinguished from the closely-related *S. Delphinii* Welch by the production of numerous, fairly small, globose sclerotia (Stevens, 1931). Curzi (1932) recognizes two species, usually termed *S. rolfsii*, on the characteristics of the hymenia, styling them *Corticium rolfsii* and *C. centrifugum*. He also finds a correlation between certain vegetative characters and the perfect stage of either species, and assumes that these characters may be employed in identification. Goto (1933, 1933*a*) finds a similar relationship between groups of characters and sporulating capacity of various Formosan strains. In these studies, however, the extent to which the composition of the substratum, temperature, and other factors influence the nature of the growth has been overlooked.

The effects of temperature, and composition of the media, on the vegetative growth of several Australian isolates, a description of the perfect stage, recorded for the first time in Australia, and the method of parasitism are set out in this paper.

I. Cultural Characters and Perfect Stage.

(*a*). *Materials and Methods.*

Eight isolates were grown on potato-dextrose agar (Riker, 1936), onion agar and carrot agar (Goto, 1933), onion-proteose-peptone agar (Mundkur, 1934), peptone-dextrose broth (peptone 35 grm., dextrose 35 grm., distilled water 1,000 ml.) and pectin broth ($MgSO_4$ 0·26 grm., K_2HPO_4 0·26 grm., citrus pectin 20 grm., distilled water 1,000 ml.). In the temperature studies, the isolates were grown on potato-dextrose agar at 15°, 20°, 25°, 30° and 37°C.

(*b*). *Observations.*

(i). *Effect of media on growth.*

Both the type and rate of growth of the mycelium depend largely on the composition of the substrate, little difference being displayed by various isolates grown on the same medium, although the lower rate of S3 (Table 1) would suggest that rate of growth is also an inherent factor. Sclerotial characters are largely inherent, being influenced only slightly by the substrate composition. On carrot agar, growth is more abundant, being thicker and denser than on the other media (Pl. v, fig. 1). The white, dense, feathery growth, similar to that described by Stevens (1931) for corn-meal agar, is in direct contrast to the thinner, straighter, less-feathery growth, with more abundant aerial mycelium, typical of the other media. Potato-dextrose agar encourages rather dense, thick mycelium, with equal development of aerial and surface mycelium, growth being more vigorous and abundant than on onion and onion-proteose-peptone agars. The

TABLE 1.
Rate of Diameter Spread of Colony at 30° C. (Mm. per 24 hours.)

Culture.	Medium. Potato-dextrose Agar.	Onion Agar.	Onion-proteose-peptone Agar.	Carrot Agar.	Mean of Culture.
S1	28	32	24	23	27
S2	28	29	28	24	27
S3	29	22	22	25	24
S4	28	28	28	28	28
S5	29	27	29	27	28
Mean for Medium	28	28	26	25	27

mycelium is more silky on onion agar, with abundant strands of aerial mycelium. It is more abundant than that produced on onion-proteose-peptone agar, which is relatively sparse and thin, and confined to the surface with very little aerial mycelium. This medium is far less suitable for the vegetative growth of the organism than any of the other solid media, but the ease with which hymenial production occurs indicates its suitability in inducing sporulation. Hymenia were not produced on any of the other media except to a very limited extent on potato-dextrose agar. Very feathery, thick, densely flocculent mycelium is produced on peptone-dextrose broth. It spreads rapidly over the surface of the broth and up the sides of the flask, contrasting with the slowly spreading, scarce mycelium on pectin broth, which is very thin and lacking in aerial mycelium. A gel was formed beneath the mycelium on this broth.

The sclerotia are little affected by the quality of the substrate, their size, shape and colour being constant on all media during the early stages of growth (i.e., during the first three weeks). There is some variation in the number produced, however, production being much less frequent on onion-proteose-peptone agar than on carrot agar, and more frequent on potato-dextrose agar than on onion agar. Their size, shape and abundance are largely inherent. Two isolates (S2 and S3) produced large, rather irregular, and relatively few sclerotia, while the remaining isolates had typically small, globose, uniform, and numerous sclerotia.

The sclerotia produced during the later stages of growth of a culture, i.e., when mycelial growth had apparently ceased and sclerotial production was progressing slowly, were usually very much larger than those produced during the earlier phases. These sclerotia were globose and of the typical brown colour, the chief variation being in size only. That this is connected with variation in the substrate—probably staling conditions—was demonstrated by growing these large sclerotia on fresh media, when the typical small types were produced in the earlier stages and larger ones produced again towards the end of the growth period.

(ii). *Effect of temperature on growth.*

Growth proceeds very slowly at 15°C., increases rapidly as the temperature is raised to 25°C., and reaches an optimum growth temperature at about 30°C. At higher temperatures it decreases rapidly, becoming very slow at 37°C. There appears to be no significant difference between the growth rates of these five isolates (Table 2). These observations are in accord with those of Higgins (1927) for several American isolates.

Considerable variation in the type of growth produced at various temperatures was noted (Pl. v, fig. 3). At 15°C. the mycelium was more sparsely distributed than at any other temperature, producing aerial mycelium far more abundantly. With higher temperatures, the production of aerial mycelium became less distinctive, giving way to a denser and more vigorous surface growth. The tendency for the sparse aerial hyphae to be lost and a more compact habit to be assumed with increased temperature was very marked, but was less apparent between 25° and 30°C. than between any other two temperatures.

TABLE 2.
Effect of Temperature on Colony Growth.

Isolate.	Increase in Diameter of Colony. (Mm. per 24 hours.)				
	15° C.	20° C.	25° C.	30° C.	37° C.
S1	5	13	24	28	6
S2	7	15	27	28	7
S3	5	11	21	29	7
S4	5	11	24	28	9
S5	4	10	21	29	4
Mean of 5 isolates	5	12	23	28	7

The time of first appearance and the number of sclerotia were also greatly affected by temperature. At 15°C. no sclerotia had appeared after fourteen days in any culture, but at the other temperatures sclerotia had appeared in all plates. Sclerotial production was more rapid at 37° than at 20°C., but less rapid than at 25° and 30°C. (Table 3).

TABLE 3.
Effect of Temperature on Sclerotial Production.

Isolate.	15° C.		20° C.		25° C.		30° C.		37° C.	
	First Appearance.	Production.*	First Appearance. (Days.)	Production.*	First Appearance. (Days.)	Production.*	First Appearance. (Days.)	Production.*	First Appearance. (Days.)	Production.*
S1	—	—	8	+	2	+++	2	++++	6	++
S2	—	—	—	—	7	++	7	++	11	+
S3	—	—	12	+	4	+++	3	++++	7	++
S4	—	—	10	+	3	+++	2	+++	4	+++
S5	—	—	11	+	4	+++	4	+++	5	+++

* + denotes relative abundance of sclerotia after 14 days.
— denotes absence of sclerotia after 14 days.

The number of sclerotia produced was greatest at 25° and 30°C., but decreased with both higher and lower temperatures, being more abundant at 37° than at 20°C.

(iii). *The perfect stage.*

During the present investigations, three isolates produced hymenia. Considerable difficulty was experienced in inducing fructification, which occurred on both onion-proteose-peptone and potato-dextrose agars, but more vigorously on the former than on the latter medium. Of various methods tried to induce sporulation, the most successful was to culture the organism on onion-proteose-peptone agar and incubate at 30°C. for two to three weeks, after which time, vegetative growth had practically ceased. The cultures were exposed to direct sunlight at room temperatures for two days, then re-incubated at 30°C. Vigorous sporulation usually followed about two weeks later. Exposure to light and maintaining the cultures at 30°C. appeared to be necessary conditions for sporulation.

In a series of cultures in which combinations of pairs of the isolates were grown under the above conditions, hymenia were only produced in those tubes containing an isolate which fructifies readily by itself. It would appear that the failure of certain isolates to fructify is not due to heterosexuality, but rather to the loss of the inherent capacity to do so, or the absence of suitable conditions.

Hymenia developed as small irregular areas, not exceeding 2 sq. mm. in extent, over the surface of the slopes. Two to fifteen separate hymenia were found in different cultures. No correlation could be found between the amount of hymenia produced and any single factor. It did not appear to depend on the capacity of the particular strain to sporulate.

Hymenia appeared as dense crustiform masses, white in colour and closely adhering to the substrate. During the early stages of development, drops of a glistening crystal-white liquid, similar to that found on the sclerotia, were exuded. The subhymenial layer consists of closely interwoven hyphae which branch monopodially. The branchlets are basally septate and rise as clavate-shaped hyphae to form the basidia. Two or more basidia frequently arise close together on the one hypha. The basidia are hyaline, club-shaped and very closely aggregated (Figs. 1*a*, *b*). Usually four sterigmata are borne at the apex, these structures tapering towards the end (Fig. 2*b*). Basidiospores arise as small spherical bodies, but they elongate and when mature are pyriform-globose in shape (Figs. 2*a* to 2*f*). They are usually apiculated at the base, and are smooth

Fig. 1.—Hymenia of *C. rolfsii*. (*a*) Immature. (*b*) Mature. × 530.
Figs. 2*a*-2*f*.—Stages in the development of the basidium of *C. rolfsii*. × 1080.

and hyaline. The dimensions of the structures were found to be constant in size for the three isolates; the basidia ranging from 2·01μ to 5·61μ (mean 4·16μ) in length and 1·67μ–3·61μ (2·47μ) in breadth, the sterigmata 1·34μ–2·57μ (1·95μ) in length, and the basidiospores 1·64μ–2·51μ (1·94μ) in length and 1·00μ–1·72μ (1·37μ) in breadth.

Curzi (1932) distinguishes two species whose vegetative stages are usually known as *Sclerotium rolfsii*:

(1). *Corticium rolfsii* (Sacc.) Curzi.—Mycelium never flocculent and mostly creeping; sclerotia numerous, relatively small, globose, scattered, not aggregated and not coalescing; form and size almost constant; hymenia dense, crustiform, adhering to substrate; basidiospores globose.

(2). *C. centrifugum* (Lév.) Curzi.—Mycelium flocculent, abundantly aerial; development usually centrifugally; sclerotia not very numerous, irregularly scattered, aggregated and coalescing; hymenia loose, never crustiform, with the basidia-bearing hyphae in white tufts, mostly aerial; basidiospores long, oval or pyriform.

The three strains which sporulated in culture (S1, S3, S4) have similar mycelial and sclerotial characters to those described for *C. rolfsii*; although on some media, as carrot agar, the mycelium is definitely flocculent. Aerial mycelium is also common, in which respect they resemble *C. centrifugum*. Strains S2 and S8 resemble *C. centrifugum* generally in vegetative habit, aerial mycelium being common, and sclerotia being less numerous, larger and irregularly scattered. As these strains were non-sporulating, it was impossible to refer them to their correct group. They resemble very closely the description given by Goto (1933) for several non-sporulating Japanese strains. The variation in type of growth produced on different media, however, makes it impossible to identify any isolate on vegetative characters. The hymenia of the sporulating strains more closely resemble *C. rolfsii* than *C. centrifugum*, although the shape of the basidiospores is slightly different. It is probable that environmental effects and strain variation will also influence these characters and a certain degree

of laxity must be allowed. It is therefore suggested that the name *Corticium rolfsii* should be applied to these Australian isolates.

(iv). *Variation in the cultures originated from basidiospores.*

Seven monobasidiosporidial cultures were obtained by the following method. A petri dish was poured with clear agar and allowed to cool. A portion of the hymenium was then placed on the lid of the dish and kept at 30°C. for one hour. The temperature and moisture conditions were found to be satisfactory for spore discharge, but during the period allowed, only relatively few spores were obtained. The discharged spores were observed under the high power objective, isolated by means of a fine-pointed harpoon, and transferred to potato-dextrose agar. The cultures were examined until germination had well proceeded to confirm the authenticity of the cultures, which were kept at 30°C. during germination and growth.

Considerable variation was noticeable in the growth habit of the cultures (designated as B1, B2, B3, B4, B5, B6, B7) both between each other and respective mother cultures (Pl. v, fig. 4). B1, B4, B5 and B7 were very similar in appearance. During the first three days of growth, they produced an abundance of fine aerial mycelium, surface or compressed growth being relatively slight. On the other hand, B2 gave rise to a mass of rather dense surface mycelium, aerial mycelium being entirely lacking. B6 produced aerial and surface growth in almost equal amounts, but the surface mycelium was finer and sparser than in B2. B3 was vastly different from the other cultures, being very slow growing and producing dense mycelium, closely confined to the surface of the medium, and with no aerial mycelium.

Variation in sclerotial production was also apparent. B1, B5 and B7 were rather similar, producing abundant, small, globose sclerotia within four days of inoculation. The sclerotia were irregularly scattered through a band on the plate about one-third the radius in width and situated towards the outer portion of the plate. B6 differed by producing a great abundance of very small, globose sclerotia scattered irregularly over the whole plate. The sclerotia were much smaller and far more numerous in this culture than in any other sporidial or mother culture. B4 formed large sclerotia, with a tendency to aggregate. In B3, the sclerotia were even larger than in B4, were very scarce, and coalesced. Sclerotia took much longer to appear in B3 and B4, being about fourteen days in B3 and seven days in B4. Growth rates for the various cultures on potato-dextrose agar at 30°C. are given in Table 4.

TABLE 4.
Increase in Mean Diameter of Colonies.
(*Mm. per 24 hours.*)

Culture.	Diameter Increase.
B1	30
B2	28
B3	10
B4	27
B5	28
B6	29
B7	32

To determine whether the cultures retained the capacity to sporulate, they were grown singly and in combination of pairs under conditions which induced sporulation in the mother cultures. Three cultures only produced hymenia, and again heterosexuality could not be demonstrated. It would appear that sporulation can be induced only under very exact conditions, and this varies with different strains, or else the capacity to fructify is readily lost. The former seems more likely.

(*c*). *Discussion and Conclusions.*

The composition of the medium upon which *C. rolfsii* is growing greatly affects its mycelial growth and the relative abundance of sclerotia, although it has little effect on the colour or shape of the sclerotia. The size of these bodies varies with the age

of the culture. Great care is therefore necessary in separating various strains (or species) on vegetative characters. Hymenial characters are considered to be the only accurate basis of classification of this group of closely related fungi, often considered as *Sclerotium rolfsii*, but the difficulty in inducing sporulation may make it necessary to consider vegetative characters as points of differentiation. If such a resort be necessary, the organism should be grown on a standard medium for a specified period and at a specified temperature. Such a classification would be tentative and preliminary to sporulation.

The wide range of cultural characters produced under artificial conditions by strains, most of which have an apparently similar host range, make it essential that any classification should be broad, allowing considerable range within the terminology of the species. Curzi's classification (1932) was used in identification in this paper. This was thought to be the best available, but whether it is adequate is yet unknown.

II. Mechanism of Parasitism.

(*a*). *Mode of Infection and Effect on Host Tissue.*

The method of entry of *C. rolfsii* was studied by inoculating hypocotyls of germinating bean seeds with small drops of a mycelial suspension prepared by macerating an actively-growing culture of the pathogen in a tube of sterile water. The inoculated hypocotyls were incubated at 30°C., and sections fixed at intervals until pathogenic action was complete. Sections were fixed in Flemming's osmic acid solution, sectioned and stained with various combinations, including safranin—light green, gentian violet—orange G, and carbol-fuchsin—light green.

(i). *Method of entry.*

The hyphae grow rapidly in the suspension drop and form a thick mat over the surface of the host (Fig. 3). From this mat, small mycelial strands grow down towards the cuticle. On reaching the cuticle, appressoria are formed, penetration taking place after the manner described by Blackman and Welsford (1916) for *Botrytis cinerea* (Figs. 4*a*, *b*, *c*, *d*). Formation of appressoria and penetration rarely precede the establishment of the mycelial mat, although this structure does not appear to have any significant function in penetration. It is more likely to be the natural outcome of ideal growth conditions.

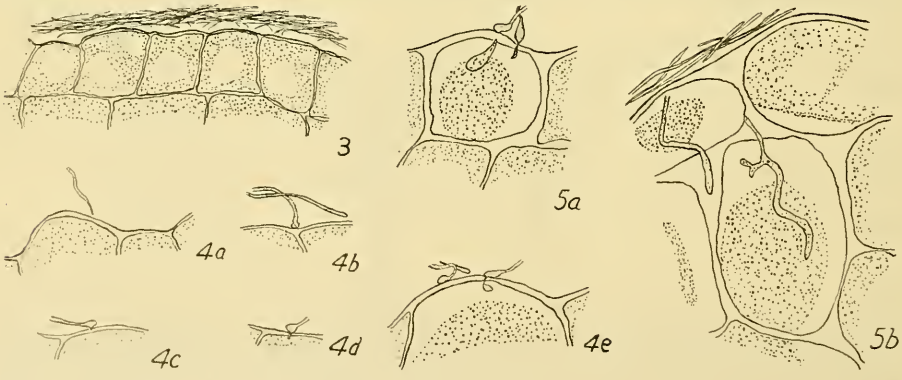

Fig. 3.—Mycelial mat growing over surface of host. × 410.
Figs. 4*a*-4*c*.—Entry of *C. rolfsii* showing appressoria and penetration hyphae. × 830.
Fig. 5.—(*a*) Growth through epidermal cells showing expansion of hyphae after entry and death of cells in advance. × 410. (*b*) Death of cells in advance and dissolution of middle lamellae. Inter- and intra-cellular hyphae. × 410.

The penetration hyphae expand on reaching the epidermal cells, assuming normal dimensions (Fig. 4*e*). There is no visible action on the host tissue until penetration has taken place, but, immediately after, plasmolysis and death of the invaded cell become visible (Fig. 5*a*). As the hyphae grow, dying of the protoplasm extends,

the protoplasm being always killed in advance of the invading hyphae, showing that some toxic substance is diffusing from the fungal mycelium.

(ii). *Growth through host tissue.*

The invading mycelium is both inter- and intra-cellular. It readily permeates the whole of the tissue, branching frequently both in the cells and in the inter-cellular spaces. The hyphae are often thickly clustered together, especially in the outer layers. Penetration of all cell walls within the tissue is mechanical, appressoria being formed. In tissue which has been invaded for several days, appressoria are very numerous and can be readily distinguished as enlarged oval structures (Figs. 7*a* and *b*). During the first stages of invasion, the hyphae are coenocytic, few cell walls being formed (Figs. 6*a* and *b*). Later, however, cell walls become more numerous (Figs. 7*a* and *b*). The hyphae are usually multinucleate during all stages of invasion. Generally the invading hyphae grow parallel to the long axis of the cell, less frequently obliquely, but rarely across the cell. No particular preference is shown by the fungus for any portion of the tissue. Growth proceeds as readily through the cells as along the inter-cellular spaces.

Death of the cytoplasm precedes hyphal invasion to a depth of usually one layer, the cells being rarely killed at a distance of more than one layer from the invading fungus. The nuclei remain normal in shape and appearance until the cells are fully

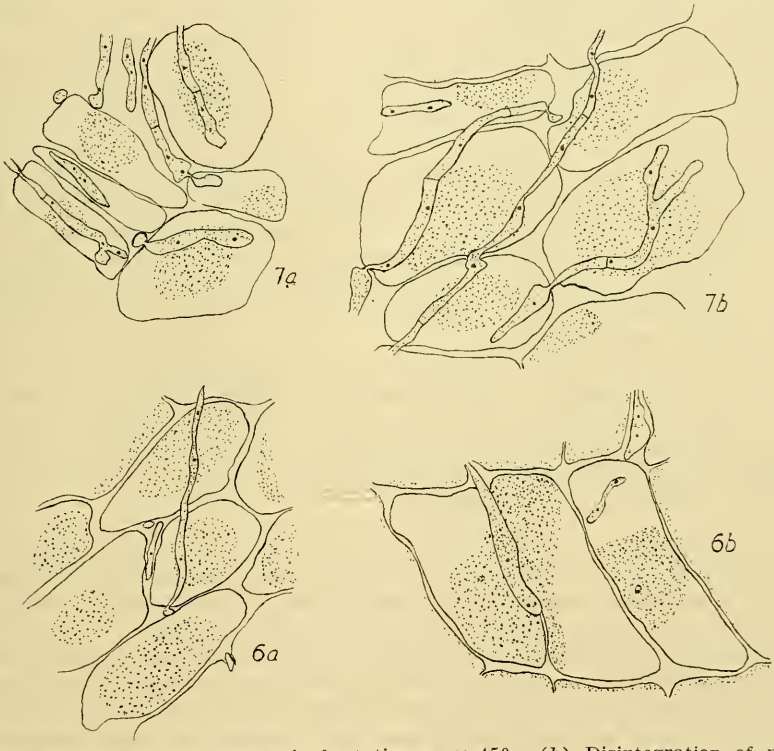

Fig. 6.—(*a*) Coenocytic hyphae in host tissue. × 450. (*b*) Disintegration of nucleus of invaded cell. × 910.

Figs. 7*a*, *b*.—Multinucleate septate hyphae, with killed and disintegrating cells. × 910.

invaded by the hyphae. Although in many cases the cytoplasm has been plasmolysed and hyphae are growing in the cell, the nuclei still remain intact (Fig. 6*a*). When the cell has been fully permeated by the hyphae, the nuclei are seen to disintegrate and disappear (Fig. 6*b*). It would seem that invasion must proceed to a marked degree before the nuclei are finally destroyed. This would not necessarily indicate, however, that their function did not cease until then, as it is highly probable that death occurs long before disintegration has taken place, as is the case with the cytoplasm.

Dissolution of the middle lamellae was evident early in the action of the fungus (Fig. 5*a*). The rapid maceration of the tissue as a result of this action would indicate the presence of a pectilytic enzyme.

(*b*). *Investigation of the Toxic Principle.*

Considerable controversy exists as to the exact nature of the offensive action of facultative parasites. Enzymes and organic acids, especially oxalic acid, are most favoured. Brown (1936) has recently reviewed the subject, showing that the bulk of the evidence favours the enzyme theory. Higgins (1927) has suggested oxalic acid as the toxic substance of *S. rolfsii*. He isolated large quantities of oxalic acid from carbohydrate broths after thirty-one days' growth. Tests with these filtrates showed that they were extremely toxic. Filtrates boiled for one minute gave positive, but less conclusive, results, which he stated indicated that the toxin was thermostable. Oxalic acid was found to have a bleaching and destructive effect in concentrations of 1:10,000. He does not indicate, however, whether the symptoms of the destructive action are similar in each case, as it is possible that oxalic acid, though toxic, may not be the substance concerned in parasitism. Besides it would seem that his cultures, due to their age, would have an oxalic acid content far greater than that shown by the fungus at the time of parasitism. No knowledge of the concentration of acid in the vicinity of invading hyphae is available, and it is a matter of conjecture whether it is produced in sufficient concentrations to account for the offensive action.

The following investigations were initiated in an attempt to obtain more conclusive evidence as to the nature of the toxic substance. The fungus was grown on pectin and peptone broths (see *Materials and Methods*) and peptone-dextrose broth (peptone 20 grm., citrus pectin 20 grm., distilled water 1,000 ml.). The broths were inoculated with small pieces of three-day-old cultures and incubated at 30°C. for three days, when the mycelium was harvested after the method described by Davison and Willaman (1927). An extract was prepared from the dry mycelium by grinding 0·5 grm. with 25 c.c. sterilized distilled water and allowed to stand for twenty-four hours at 40°C., toluene being added to prevent bacterial activity. (Toluene does not affect enzyme action.) All fragments of the mycelial powder were removed by centrifuging and the clear extract used immediately.

All pieces of mycelium were removed from the broths upon which the fungus was growing by filtering through thinly-woven linen. Filter papers were not used as it was considered they might absorb the enzyme. Toluene was added to the filtrates. Half of each filtrate was used for determining direct macerating effects, the remaining portion being used for enzyme precipitation with equal volumes of 95% alcohol. The precipitate was removed by centrifuging.

(i). *Macerating effect.*

The macerating effects of both filtrate and mycelial extracts, unheated and after steaming for one hour, were tested by their action on potato tissue. Small uniform strips of potato tuber were cut 0·5 mm. in thickness with a razor. Controls were run with sterile water, sterile water plus toluene, sterile broths (i.e., broths upon which no fungus had been growing), and sterile broths plus toluene. Solutions of oxalic acid in concentrations of 1:100, 1:1,000, 1:5,000, 1:10,000 were also tested. Total maceration, the point of ready disintegration of the tissue, was taken as the end-point. The result was judged purely qualitatively by the fingers, but accurate comparative results were obtained.

Total maceration was obtained in the mycelial extracts from pectin broth in six hours, from peptone-pectin broth in eight hours, and from peptone-dextrose broth in twelve hours, and from all three filtrates in twelve hours; no maceration was obtained in the heated filtrates and mycelial extracts, or the controls within five days. Oxalic acid totally macerated potato tissue in sixty hours when in concentrations of 1:100, but in concentrations of 1:1,000 or greater no action was observed within five days.

The fungus produces a thermolabile substance, capable of rapidly macerating plant tissue, and which differs greatly from oxalic acid both in rate of action and in effects

produced. It corresponds to the enzyme described by Davison and Willaman (1927) as protopectinase.

(ii). *Pectase determination.*

To determine the presence of pectase, tests were carried out with filtrates, mycelial extracts, and solutions of the re-dissolved alcoholic precipitates. Two methods were used, the coagulation method of Davison and Willaman (1927) and the calcium mono-methyl tartrate method (Thornberry, 1938). Negative results were obtained in all cases.

(iii). *Pectinase determination.*

The presence of pectinase was determined by Davison and Willaman's method (1927), the reducing sugars obtained being estimated by the picric acid method (Willaman and Davison, 1924). Mycelial extracts from 0·5 grm. mycelial mat and the solutions of alcoholic precipitate obtained from 10 ml. filtrate were used in the determinations. The results are given in Table 5, the amount of reducing sugar expressed

TABLE 5.
Reducing Effect of Fungal Solutions on Pectin.

Solution.	Reducing Sugars in Solution after 24 hours at 40° C.
Mycelial extracts from :	
Peptone-dextrose broth	2·57%
Peptone-pectin broth	2·71%
Pectin broth	2·90%
Alcoholic precipitates from :	
Peptone-dextrose broth	1·80%
Peptone-pectin broth	2·02%
Pectin broth	2·31%
Controls :	
Peptone-dextrose broth	0·04%
Peptone-pectin broth	0·06%
Pectin broth	0·09%
Alcoholic precipitates from :	
Peptone-dextrose broth	0·00%
Peptone-pectin broth	0·01%
Pectin broth	0·01%

being that obtained from the hydrolysis of 20 ml. 3% pectin solution. Controls were run by omitting the pectin, which had no reducing effect.

The enzyme pectinase is produced by *C. rolfsii*, being produced in greater quantities on a pectic medium than on a peptone-dextrose medium.

(c). *Discussion and Conclusions.*

Both protopectinase and pectinase are produced by *C. rolfsii* and are responsible for its destructive action on plant tissue. Although much confusion exists at present as to the nature and effects of pectilytic enzymes, yet the main point that the action is enzymic has been established. Oxalic acid is certainly not responsible. The discrepancy in Higgins's (1927) results is probably due to the very high concentrations of oxalic acid present in the old cultures used. Boiling the solutions for one minute may be insufficient to coagulate all the enzyme in the solution. Steaming for an hour, however, appears to irreversibly precipitate the enzyme. His results also show a more delayed and less effective action with the boiled filtrates, indicating that the toxic action may be somewhat reduced after boiling for so short a period as one minute.

It must be remembered that oxalic acid has a macerating action in high concentrations, but it is difficult to imagine how such concentrations could be obtained during parasitism, as the action of *C. rolfsii* is so rapid. The demonstration of pectilytic

enzymes, however, offers a more feasible explanation for the rapidity and effectiveness of this action.

Summary.

Part i.—Studies on eight isolates of the organism formerly known as *Sclerotium rolfsii* showed that the abundance and type of vegetative growth are determined largely by the nature of the medium and temperature. Abundance and size of the sclerotia, but not shape or colour, are also affected by these factors.

The optimum temperature for growth is 30°C., and this decreases markedly to 15°C. and to 37°C.

The hymenial stage is reported for the first time in Australia, and is designated *Corticium rolfsii* (Sacc.) Curzi.

Distinct variations in the growth habit of seven monobasidiosporidial cultures are also noted.

Part ii.—The mechanism of the parasitic action of *C. rolfsii* is discussed. It penetrates plant tissue mechanically, and gives rise to coenocytic intra- and inter-cellular hyphae. Death of the cytoplasm precedes the invading hyphae by one cell layer. Dissolution of the middle lamellae occurs shortly after entry.

Protopectinase and pectinase, but not pectase, are produced and are responsible for its toxic action.

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EXPLANATION OF PLATE V.

1.—Growth of isolate S3 on potato-dextrose agar (P.D.A.), carrot agar (C.A.), onion-proteose-peptone agar (O.P.P.A.), and onion agar (O.A.) at 30°C.

2.—Vegetative growth of different isolates on potato-dextrose agar at 30°C.

3.—Growth on potato-dextrose agar at different temperatures after three days.

4.—Variations in growth of basidiosporidial cultures on potato-dextrose agar at 30°C., with cultures from which they were derived.